

Amendments to the Claims

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of the Claims:

1-56 (Canceled).

57 (Currently amended): A method for loading active agents into liposomes comprising:

- a) providing a liposome comprising a gel-phase lipid bilayer and a liposome interior defined by the gel-phase lipid bilayer, said gel phase lipid bilayer comprising phospholipid, wherein said gel-phase lipid bilayer is present below its phase transition temperature; and
- b) exposing the gel-phase lipid bilayer to an active agent such that said active agent passes into and through the gel-phase lipid bilayer to load the liposome interior with the active agent.

58 (Canceled).

59 (Original): A method according to claim 57 wherein the liposome is present in a surrounding liquid medium, and wherein the pH of the surrounding liquid medium is greater than the pH of the interior of the liposome to facilitate loading of the active agent.

60 (Original): A method according to claim 57 wherein the lipid bilayer further comprises a surface active agent.

61 (Previously presented): A method according to claim 60 wherein the surface active agent is selected from the group consisting of myristoyl surfactants, palmitoyl surfactants, stearoyl surfactants, polyethylene glycol-derivatized surfactants, glyceryl monopalmitate, glyceryl monooleate, ceramides, PEG-ceramides, polyethylene glycol-polyethylene copolymers, C-18 ether linked lysophosphatidyl choline, and mixtures thereof.

62 (Original): A method according to claim 60 wherein the surface active agent is lysolipid.

63 (Original): A method according to claim 62 wherein the lysolipid is selected from the group consisting of monopalmitoylphosphatidylcholine (MPPC), monolaurylphosphatidylcholine (MLPC), monomyristoylphosphatidylcholine (MMPC), monostearoylphosphatidylcholine (MSPC), and mixtures thereof.

64 (Original): A method according to claim 60, wherein said phospholipid is dipalmitoylphosphatidylcholine (DPPC) and said surface active agent is lysolipid which is monopalmitoylphosphatidylcholine (MPPC).

65 (Canceled).

66 (Previously presented): A liposome, comprising: an active agent and a liposome interior defined by a gel-phase bilayer membrane, wherein the gel-phase bilayer has a phase transition temperature of 39 to 45°C, and

wherein the gel-phase lipid bilayer membrane comprises:

- (a) a first component which is one or more phospholipids selected from the group consisting of phosphatidyl cholines, phosphatidyl glycerols, phosphatidyl inositol, phosphatidyl ethanolamines, and sphingomyelins, wherein one or more phospholipids have two acyl groups; and
- (b) a second component selected from:
 - (i) one or more surface active agents selected from the group consisting of lysolipids, bile acids, myristoyl surfactants, palmitoyl surfactants, stearoyl surfactants, glyceryl monooleate, ceramides, PEG-ceramides, C18-ether linked lysophosphatidyl choline, polyethylene glycol-polyethylene copolymers, fatty acids, and mixtures thereof; or
 - (ii) the active agent, wherein the active agent is selected from the group consisting of a pharmacologically active agent, a flavor agent, or a diagnostic agent, or a nutritional agent; and,
- (c) wherein the active agent, if absent from the gel-phase lipid bilayer membrane, is present in the liposome interior; and
- (d) wherein the amount of the second component in the gel-phase lipid bilayer membrane is sufficient to increase a first percentage of active agent released from the liposome at the phase transition temperature, compared to a second percentage of active agent released in the absence of the second component.

67 (Previously presented): The liposome according to claim 66, wherein the second component is the active agent.

68 (Previously presented): The liposome according to claim 67, wherein the active agent is a pharmacologically active agent selected from the group consisting of ceramides and platelet activating factor.

69 (Currently amended): The liposome according to claim 66, wherein the active agent material is within the liposome interior.

70 (Currently amended): A method of administering an active agent to a preselected target site in a subject's body, comprising:

- (a) administering a liposome to the subject, wherein the liposome comprises a gel-phase lipid bilayer membrane having a phase transition temperature and a liposome interior defined by the gel-phase bilayer, and an active agent, wherein the gel-phase lipid bilayer membrane comprises:
 - (i) a first component which is one or more phospholipids selected from the group consisting of phosphatidyl cholines, phosphatidyl glycerols, phosphatidyl inositols, phosphatidyl ethanolamines, and sphingomyelins, wherein the one or more phospholipids have two acyl groups; and
 - (ii) a second component selected from:
 - (I) one or more surface active agents selected from the group consisting of lysolipids, bile acids, myristoyl surfactants, palmitoyl surfactants, stearoyl surfactants, glyceryl monooleate,

ceramides, PEG-ceramides, C18-ether linked lysophosphatidyl choline, polyethylene glycol-polyethylene copolymers, fatty acids, and mixtures thereof; or

(II) the active agent, wherein the active agent is selected from the group consisting of a pharmacologically active agent, a flavor agent, or a diagnostic agent, or a nutritional agent; and,

(iii) wherein the active agent, if absent from the gel-phase lipid bilayer membrane is present in the liposome interior; and

(iv) wherein the amount of the second component in the gel-phase lipid bilayer membrane is sufficient to increase a first percentage of active agent released from the liposome at the phase transition temperature, compared to a second percentage of active agent released in the absence of the second component; and

(b) heating the subject's preselected target site to a temperature of about 39°C to 45°C to release the active agent from the liposome at the target site.

71 (Previously presented): The method of claim 70, wherein the preselected target site comprises tumor tissue.

72 (Previously presented): The method of claim 70, wherein the second component is the active agent.

73 (Currently amended): The method of claim 70 [[72]], wherein the liposome second component comprises from about 1 to about 50 mole percent of the second component.

74 (Currently amended): The method of claim 73, wherein the liposome second component comprises from about 1 to about 30 mole percent of the second component.

75 (Previously presented): The method of claim 72, wherein the active agent is selected from the group consisting of apoptopic agents and platelet activating factor.

76 (Previously presented): The method according to claim 63, wherein the surface active agent is monostearoylphosphatidylcholine (MSPC).

77 (Currently amended): The liposome according to claim 67 [[66]], wherein the active agent material is within the liposome interior and in the gel-phase lipid bilayer membrane.

78 (Currently amended): The method of claim 72, wherein the active agent material is within the liposome interior and in the gel-phase lipid bilayer membrane.

79 (New): The method of claim 57, wherein the liposome interior is loaded with a therapeutic dose of the active agent.

80 (New): The method of claim 79, wherein the liposome interior is loaded with about 0.01M to about 10M active agent.

81 (New): The method of claim 80, wherein the liposome interior is loaded with about 0.05M to about 5M active agent.

82 (New): The method of claim 81, wherein the liposome interior is loaded with about 0.05M to about 5M doxorubicin.

83 (New): The liposome of claim 66, wherein said second component is one or more bile acids.

84 (New): The liposome of claim 66, wherein said second component is one or more myristoyl surfactants.

85 (New): The liposome of claim 66, wherein said second component is one or more palmitoyl surfactants.

86 (New): The liposome of claim 66, wherein said second component is one or more stearoyl surfactants.

87 (New): The liposome of claim 66, wherein said second component is glyceryl monooleate.

88 (New): The liposome of claim 66, wherein said second component is one or more ceramides.

89 (New): The liposome of claim 66, wherein said second component is one or more PEG-ceramides.

90 (New): The liposome of claim 66, wherein said second component is a C18-ether linked lysophosphatidyl choline.

91 (New): The liposome of claim 66, wherein said second component is one or more polyethylene glycol-polyethylene copolymers.

92 (New): The liposome of claim 66, wherein said second component is one or more fatty acids.

93 (New): A liposome, comprising: an active agent and a liposome interior defined by a gel-phase bilayer membrane, wherein the gel-phase bilayer has a phase transition region having an onset temperature, a peak transition temperature and an end point temperature, and wherein the gel-phase lipid bilayer membrane comprises:

- (a) a first component which is one or more phospholipids selected from the group consisting of phosphatidyl cholines, phosphatidyl glycerols, phosphatidyl inositol, phosphatidyl ethanolamines, and sphingomyelins, wherein one or more phospholipids have two acyl groups; and

(b) a second component selected from:

- (i) one or more surface active agents selected from the group consisting of dichain phospholipids, lysolipids, bile acids, myristoyl surfactants, palmitoyl surfactants, stearoyl surfactants, ceramides, PEG-ceramides, PEG-phospholipids, C18-ether linked lysophosphatidyl choline, block copolymers, fatty acids, and mixtures thereof; or
 - (ii) the active agent, wherein the active agent is selected from the group consisting of a pharmacologically active agent, a flavor agent, or a diagnostic agent, or a nutritional agent; and,
- (c) wherein the active agent, if absent from the gel-phase lipid bilayer membrane, is present in the liposome interior; and
- (d) wherein the amount of the second component in the gel-phase lipid bilayer membrane is sufficient to increase a first percentage of active agent released from the liposome at the phase transition region, compared to a second percentage of active agent released at the transition region of a liposome comprising only the first component.

94 (New): The liposome of claim 93, wherein said second component is one or more dichain phospholipids.

95 (New): The liposome of claim 94, wherein said dichain phospholipids comprise acyl chains of less than 12 carbons.

96 (New): The liposome of claim 94, wherein said dichain phospholipids are saturated phospholipids.

97 (New): The liposome of claim 93, wherein said second component is one or more lysolipids.

98 (New): The liposome of claim 97, wherein said lysolipid is selected from the group consisting of monopalmitoylphosphatidylcholine (MPPC), monolauryl-phosphatidylcholine (MLPC), monomyristoylphosphatidylcholine (MMPC), monostearoyl-phosphatidylcholine (MSPC), and mixtures thereof.

99 (New): The liposome of claim 98, wherein said lysolipid is monopalmitoylphosphatidylcholine (MPPC).

100 (New): The liposome of claim 98, wherein said lysolipid is monostearoyl-phosphatidylcholine (MSPC).

101 (New): The liposome of claim 98, wherein said lysolipid is monopalmitoylphosphatidylcholine (MPPC) and said first component is dipalmitoylphosphatidylcholine (DPPC).

102 (New): The liposome of claim 98, wherein said lysolipid is monostearoylphosphatidylcholine (MSPC) and said first component is dipalmitoylphosphatidylcholine (DPPC).

103 (New): The liposome of claim 102, wherein said DPPC and said MSPC are contained in a molar ratio from about 99:1 to about 70:30.

104 (New): The liposome of claim 93, wherein said second component is one or more bile acids.

105 (New): The liposome of claim 93, wherein said second component is one or more myristoyl surfactants.

106 (New): The liposome of claim 93, wherein said second component is one or more palmitoyl surfactants.

107 (New): The liposome of claim 93, wherein said second component is one or more stearoyl surfactants.

108 (New): The liposome of claim 93, wherein said PEG-phospholipid is a PEG-phosphatidylethanolamine.

109 (New): The liposome of claim 93, wherein said second component is one or more ceramides.

110 (New): The liposome of claim 93, wherein said second component is one or more PEG-ceramides.

111 (New): The liposome of claim 93, wherein said second component is a C18-ether linked lysophosphatidyl choline.

112 (New): The liposome of claim 93, wherein said second component is one or more block copolymers.

113 (New): The liposome of claim 93, wherein said second component is one or more fatty acids.

114 (New): The liposome of claim 93, wherein said second component is the active agent.

115 (New): The liposome of claim 114, wherein said active agent is a pharmacologically active agent selected from the group consisting of ceramides and platelet activating factor.

116 (New): The liposome of claim 93, having a diameter of from about 50 nanometers to about 400 nanometers.

117 (New): The liposome of claim 93, wherein said one or more phospholipids have two acyl chains each comprising 16 to 24 carbon atoms.

118 (New): The liposome of claim 93, wherein said one or more phospholipids have two acyl chains each comprising 18 to 22 carbon atoms.

119 (New): The liposome of claim 93, wherein said gel-phase lipid bilayer further comprises a phospholipid derivatized with a hydrophilic polymer.

120 (New): The liposome of claim 119, wherein said hydrophilic polymer is selected from the group consisting of polyethylene glycol, polylactic acid, polyglycolic acid, copolymers of polylactic acid and polyglycolic acid, polyvinyl alcohols, polyvinylpyrrolidone, oligosaccharides, and mixtures thereof.

121 (New): The liposome of claim 93, wherein said active agent is a pharmacologically active agent selected from the group consisting of anesthetics, antihistamines, antineoplastics, anti-ulceratives, anti-seizure agents, muscle relaxants, immunosuppressive agents, anti-infective agents, non-steroidal anti-inflammatory agents, imaging agents, nutritional agents, and mixtures thereof.

122 (New): The liposome of claim 121, wherein said active agent is selected from the group consisting of antineoplastic agents, non-steroidal anti-inflammatory agents, immunosuppressive agents, and anti-infective agents.

123 (New): The liposome of claim 121, wherein said active agent is an antihistamine.

124 (New): The liposome of claim 121, wherein said active agent is an antineoplastic agent or an antitumor agent.

125 (New): The liposome of claim 121, wherein said active agent is selected from the group consisting of methotrexate, doxorubicin, epirubicin, daunorubicin, vincristine, vinblastine, etoposide, ellipticine, camptothecin, paclitaxel, docetaxol, cisplatin, prednisone, methylprednisone, and navalbene.

126 (New): The liposome of claim 125, wherein said active agent is paclitaxel.

127 (New): The liposome of claim 125, wherein said active agent is camptothecin.

128 (New): The liposome of claim 125, wherein said active agent is doxorubicin.

129 (New): The liposome of claim 125, wherein said active agent is a non-steroidal anti-inflammatory agent.

130 (New): The liposome of claim 129, wherein said active agent is ibuprofen.

131 (New): The liposome of claim 93, wherein said active agent is within the liposome interior.

132 (New): The liposome of claim 93, wherein the acyl groups of the phospholipid are saturated.

133 (New): The liposome of claim 93, wherein said phospholipid and said second component are contained in a molar ratio of from about 99:1 to about 51:49.

134 (New): The liposome of claim 133, wherein said phospholipid and said second component are contained in a molar ration of from about 99:1 to about 70:30.

135 (New): The liposome of claim 134, wherein said second component is one or more lysolipids.

136. (Previously presented): The liposome of claim 93, wherein said active agent is within the liposome interior and in the gel-phase lipid bilayer membrane.

137 (New): A method of administering an active agent to a preselected target site in a subject's body, comprising:

(a) administering a liposome to the subject, wherein the liposome comprises:

a gel-phase lipid bilayer membrane having a phase transition region having an onset temperature, a peak transition temperature and an end point temperature;

a liposome interior defined by the gel-phase bilayer;

and an active agent,

wherein the gel-phase lipid bilayer membrane comprises:

- (i) a first component which is one or more phospholipids selected from the group consisting of phosphatidyl cholines, phosphatidyl glycerols, phosphatidyl inositols, phosphatidyl ethanolamines, and sphingomyelins, wherein the one or more phospholipids have two acyl groups; and
- (ii) a second component selected from:
 - (I) one or more surface active agents selected from the group consisting of dichain phospholipids, lysolipids, bile acids, myristoyl surfactants, palmitoyl surfactants, stearoyl surfactants, ceramides, PEG-ceramides, PEG-phospholipids, C18-ether linked lysophosphatidyl choline, block copolymers, fatty acids, and mixtures thereof; or
 - (II) the active agent, wherein the active agent is selected from the group consisting of a pharmacologically active agent, a flavor agent, or a diagnostic agent, or a nutritional agent; and,
- (iii) wherein the active agent, if absent from the gel-phase lipid bilayer membrane is present in the liposome interior; and

(iv) wherein the amount of the second component in the gel-phase lipid bilayer membrane is sufficient to increase a first percentage of active agent released from the liposome at the phase transition region, compared to a second percentage of active agent released at the transition region of a liposome comprising only the first component; and

(b) heating the subject's preselected target site to a temperature of about 38°C to 45°C to release the active agent from the liposome at the target site.

138 (New): The method of claim 137, wherein the preselected target site comprises tumor tissue.

139 (New): The method of claim 137, wherein the liposome comprises an antineoplastic agent.

140 (New): The method of claim 139, wherein the antineoplastic agent is selected from the group consisting of methotrexate, doxorubicin, epirubicin, daunorubicin, vincristine, vinblastine, etoposide, ellipticine, camptothecin, paclitaxel, docetaxol, cisplatin, prednisone, methyl-prednisone, and navalbene.

141 (New): The method of claim 137, wherein said administration in (a) comprises intravenous administration.

142 (New): The method of claim 137, wherein the acyl groups of the phospholipid are saturated.

143 (New): The method of claim 137, wherein the liposome comprises from about 1 to about 50 mole percent of the second component.

144 (New): The method of claim 143, wherein the liposome comprises from about 1 to about 30 mole percent of the second component.

145 (New): The method of claim 137, wherein the second component is one or more lysolipids.

146 (New): The method of claim 145, wherein the lysolipids are selected from the group consisting of monopalmitoylphosphatidylcholine (MPPC), monolaurylphosphatidylcholine (MLPC), monomyristoylphosphatidylcholine (MMPC), monostearoylphosphatidylcholine (MSPC), and mixtures thereof.

147 (New): The method of claim 137, wherein the active agent is entrapped within the interior of the liposome.

148 (New): The method of claim 137, wherein the active agent is entrapped within the gel-phase lipid bilayer membrane of the liposome.

149 (New): The method of claim 137, wherein the liposome has a diameter of from about 50 nanometers to about 400 nanometers.

150 (New): The method of claim 137, wherein the gel-phase lipid bilayer membrane further comprises a phospholipid derivatized with a hydrophilic polymer.

151 (New): The method of claim 150, wherein the hydrophilic polymer is selected from the group consisting of polyethylene glycol, polylactic acid, polyglycolic acid, copolymers of polylactic acid and polyglycolic acid, polyvinyl alcohols, polyvinylpyrrolidone, oligosaccharides, and mixtures thereof.

152 (New): The method of claim 137, wherein the active agent is a pharmacologically active agent selected from the group consisting of anesthetics, antihistamines, antineoplastics, anti-ulceratives, anti-seizure agents, muscle relaxants, immunosuppressive agents, anti-infective agents, non-steroidal anti-inflammatory agents, imaging agents, nutritional agents, and mixtures thereof.

153 (New): The method of claim 152, wherein the active agent is selected from the group consisting of antineoplastic agents, non-steroidal anti-inflammatory agents, immunosuppressive agents, and anti-infective agents.

154 (New): The method of claim 152, wherein the active agent is an antihistamine.

155 (New): The method of claim 152, wherein the active agent is a non-steroidal anti-inflammatory agent.

156 (New): The method of claim 155, wherein the active agent is ibuprofen.

157 (New): The method of claim 137, wherein the second component is the active agent.

158 (New): The method of claim 157, wherein the active agent is selected from the group consisting of apoptotic agents and platelet activating factor.

159 (New): The method of claim 137, wherein the active agent is within the liposome interior and in the gel-phase lipid bilayer membrane.

160 (New): The method of claim 146, wherein the lysolipid is monostearoylphosphatidylcholine (MSPC).

161 (New): The method of claim 159, wherein the phospholipid is dipalmitoylphosphatidylcholine (DPPC).

162 (New): The liposome of claim 93, wherein said phase transition region has an onset temperature of about 38°C.

163 (New): The liposome of claim 93, wherein said phase transition region has a peak transition temperature of about 38°C to about 45°C.

164 (New): The method of claim 137, wherein said phase transition region has an onset temperature of about 38°C.

165 (New): The liposome of claim 137, wherein said phase transition region has a peak transition temperature of about 38°C to about 45°C.

166 (New): A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and one or more liposomes of claim 93.

167 (New): The pharmaceutical composition of claim 166, wherein the liposomes are dispersed in an aqueous solution.

168 (New): The pharmaceutical composition of claim 167, comprising physiological saline or phosphate buffered physiological saline.

169 (New): A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and one or more liposomes of any one of claims 94-136.

170 (New): The liposome of claim 66 or claim 93, wherein said second component is present in both an inner monolayer and an outer monolayer of said gel phase bilayer.

171 (New): The liposome of claim 66 or claim 93, wherein said second component is present in only an inner monolayer of said gel phase bilayer.

172 (New): The liposome of claim 66 or claim 93, wherein said second component is present in only an outer monolayer of said gel phase bilayer.

173 (New): The method of claim 70 or claim 137, wherein the second component is present in both an inner monolayer and an outer monolayer of the gel phase bilayer.

174(New): The method of claim 70 or claim 137, wherein the second component is present in only an inner monolayer of the gel phase bilayer.

175 (New): The method of claim 70 or claim 137, wherein the second component is present in only an outer monolayer of the gel phase bilayer.